A New Species of Woodland Salamander of the *Plethodon glutinosus* Group from the Southern Appalachian Mountains

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ABSTRACT.— A new species of woodland salamander, *Plethodon aureolus,* occurs between the Little Tennessee and Hiwassee rivers on the western slopes of the Unicoi Mountains and nearby lowlands in southeastern Tennessee and adjacent North Carolina. It is a small-sized member of the *P. glutinosus* group and was discovered by a study of electrophoretic variation in 22 genetic loci. It is sympatric with the white-spotted form of *P. glutinosus* (here recognized as a distinct species, *P. teyahalee)* at 28 localities, and at one of these it is also sympatric with typical brassy-spotted *P. glutinosus. Plethodon aureolus* hybridizes with Unicoi Mountain *P. jordani* on Sassafras Ridge, but there is no evidence of significant hybridization between *P. aureolus* and *P. teyahalee* or *P. glutinosus.*

Two unpublished electrophoretic studies of geographic genetic variation in eastern woodland salamanders of the *Plethodon glutinosus* group, one by Peabody (1978) and the other in preparation by Highton, have revealed the existence of an undescribed species of the group. Its range appears to be largely restricted to the western slopes of the Unicoi Mountains and adjacent lowlands, between the Little Tennessee and Hiwassee rivers, in Monroe and northern Polk counties, Tennessee, and adjacent Graham and Cherokee counties, North Carolina.

Highton (1970) called attention to the presence of three distinct geographically parapatric color pattern variants of *P. glutinosus* in the southern Appalachian Mountain region: (1) populations in the mountains of western North Carolina are characterized by having small dorsal white spots; (2) in populations from northeastern Georgia many individuals lack dorsal spotting; and (3) populations to the west and south of the above areas are characterized by having brassy-colored dorsal spots. Highton (1972) mapped the distribution of three parapatric dorsal pattern variants of *P. glutinosus* in Pennsylvania, Maryland, Virginia and West Virginia. Two of these resemble the first and the third southern Appalachian types in the color of their dorsal spots, while a third, smaller, Coastal Plain variant is characterized by its very small dorsal brassy-colored spots. I suggested that there may be limited gene exchange between some of these parapatric forms and some pairs may be at or close to the species level of evolutionary divergence. Our unpublished genetic studies have shown that hybridization often occurs

in the narrow overlap zones where the ranges of some of the above parapatric forms are in contact. The species described here differs genetically from all of the above types and throughout its range it is sympatric with the white-spotted southern Appalachian variant of *P. glutinosus*. The two forms appear to be both morphologically and genetically distinct at all 28 localities where they have been taken sympatrically.

The new species is characterized by possessing abundant brassy-colored dorsal spots and by its small size. It is very distinct from the sympatric, white-spotted, large-sized populations of *P. glutinosus,* but some other nearby brassy-spotted populations of *P. glutinosus* are very similar to it in appearance. Using the same electrophoretic methods and genetic loci described in Highton and MacGregor (1983), the new species was compared genetically to 11 samples of *P. kentucki,* 128 samples of *P. glutinosus* and 41 samples of *P. jordani* taken from localities scattered throughout their ranges. It was also compared with a single sample of each of the other four species of the group (*yonahlossee, caddoensis, ouachitae* and *fourchensis*). Geographic genetic variation in the latter three species was studied by Duncan and Highton (1979) and the remaining results are being prepared for publication. The new species is genetically distinguishable from samples of all of these species, just as it is from sympatric *P. glutinosus*. However, I have failed to find any morphological characters that may be used to distinguish it from some allopatric types of brassy-spotted *P. glutinosus*. The diagnosis presented here is therefore valid only for comparisons with the three forms of eastern large *Plethodon* with which it is sympatric. This is the second cryptic species of *Plethodon* discovered by electrophoretic studies of genetic variation in proteins, the first being *P. websteri* Highton (1979).

The new species is named for its brightly-colored brassy dorsal spots. The name is from the Latin word meaning gilded, ornamented or very beautiful.

Plethodon aureolus, new species

Diagnosis.— An eastern *Plethodon* of the *P. glutinosus* group (Highton and Larson 1979). It differs from sympatric white-spotted *P. glutinosus* by its smaller size, its relatively larger dorsal spots, the presence of abundant brassy flecking in the dorsal iridophore spots, and more abundant lateral white or yellow spotting. It differs from most nearby populations of brassy-spotted *P. glutinosus* by its smaller size and lighter chin. It differs from Unicoi Mountain *P. jordani* by the presence of dorsal spots and by its more abundant white iridophore spotting on the sides and legs.

Holotype.— USNM 238341, an adult male collected at Farr Gap (locality 1, Table 1), Unicoi Mountains, Monroe County, Tennessee, on 30 June 1979, by Richard Highton and Jeffrey K. Streicher.

Paratypes.— USNM 238342-51, topotypes, same collecting data as the holotype.

Other material.— *Plethodon aureolus* from 31 localities (Table 1) have been identified electrophoretically, and preserved specimens from all of these sites will be deposited in the National Museum of Natural History (USNM).

Description of holotype.— Before preservation, the length from the tip of the snout to the anterior angle of the vent was 54 mm, to the posterior angle of the vent 58 mm, and the total length 122 mm. There are 16 costal grooves (equivalent to 17 trunk vertebrae) and the vomerine teeth number 8 on the right side and 9 on the left. In life there were abundant dorsal white iridophore spots with much associated brassy flecking scattered on a black ground color. Similar spots were also present on the dorsal surfaces of the legs and the top of the head. There were abundant yellow iridophore spots on the sides of the head and body and a few yellow iridiphore spots were also present on the chin and belly. The chin is lighter than the belly.

Distribution.— *Plethodon aureolus* is known from southern and eastern Monroe and northeastern Polk counties, Tennessee, and also occurs in northwestern Cherokee and western Graham counties, North Carolina (Fig. 1).

Variation in P. aureolus.— There is little morphological variation in *P. aureolus* throughout its small range, except that at higher elevations in the northeastern part of its range the dorsal spotting may be very reduced or absent in some individuals.

Remarks.— Although I had collected several *P. aureolus* during my earlier study of variation in southern Appalachian large *Plethodon* (Highton 1970), it was not recognized as distinct from other brassy-spotted *P. glutinosus* until recently when we obtained new material for our electrophoretic studies. The results have indicated that the large-sized, brassy-spotted, dark-chinned populations from eastern Tennessee are closely related to *P. glutinosus* from the northern part of its range (New York west to Illinois and south through Kentucky, West Virginia, western Virginia and eastern Tennessee). Since the type locality (Princeton, New Jersey) of *P. glutinosus* is within this area, this form will retain the name *P. glutinosus* regardless of the eventual taxonomic status of the other geographic variants. These northern populations of *P. glutinosus* are characterized by possessing much darker chins than those of the white-spotted populations (Highton 1962), *P. kentucki* (Highton and MacGregor 1983) and *P. aureolus.* However, in the immediate vicinity of the range of *P. aureolus,* many individuals of otherwise genetically typical *P. glutinosus* possess unusually light chins, making it very difficult to distinguish the two species without an analysis of their proteins. The two have been found sympatrically only at locality 4, along

Table 1. List of localities and number of each species evaluated electrophoretically.

Locality No.	State	County	Elevation (m)	North latitude			West longitude			*P. aureolus*	*P. glutinosus*	*P. teyahalee*	*P. jordani*
				°	′	″	°	′	″				
1	TN	Monroe	872	35	27	45	84	01	37	36		39	
2	"	"	1128	35	21	20	84	04	42	30		32	
3	"	Polk	299	35	11	33	84	29	43	33		1	
4	"	"	293	35	15	03	84	27	20	5	6	17	
5	"	Blount	549	35	38	20	83	44	52		30		
6	"	Meigs	244	35	30	37	84	47	12		5		
7	"	Monroe	622	35	17	09	84	29	21		28		
8	"	Polk	604	35	09	15	84	36	27		32		
9	NC	Cherokee	506	35	07	19	84	17	48		30		
10	NJ	Union	91	40	40	42	74	23	10		30		
11	TN	Monroe	665	35	19	31	84	05	08	2		27	
12	NC	Graham	1116	35	23	33	83	46	26			35	
13	"	"	854	35	15	40	83	56	42				30
14	"	"	1412	35	19	57	84	01	38				36
15	TN	Monroe	268	35	32	13	84	08	09	8		2	
16	"	"	290	35	30	17	84	08	52	5		2	
17	"	"	299	35	30	07	84	06	50	3		1	
18	"	"	372	35	25	52	84	10	02	10			
19	"	"	552	35	25	33	84	03	44	4		12	
20	"	"	415	35	25	16	84	11	12	5		1	
21	"	"	323	35	24	02	84	14	57	5		1	
22	TN-NC	Monroe-Graham	1375	35	22	49	84	00	34	8		1	
23	TN	Monroe	1348	35	22	33	84	00	50	20		1	
24	"	"	1201	35	21	36	84	06	08	30		5	
25	"	"	354	35	19	56	84	18	27	7		3	
26	"	"	500	35	19	46	84	08	47	3		1	
27	"	"	451	35	19	26	84	10	37	20			
28	"	"	598	35	19	17	84	07	13	1		3	

29	"	"	537	35	19	15	84	08	50	2	1
30	"	"	320	35	17	44	84	24	03	10	1
31	"	"	1012	35	16	18	84	13	15	2	2
32	TN-NC	Monroe-Cherokee	988	35	16	03	84	13	15	5	3
33	TN	Polk	348	35	14	47	84	27	01	28	1
34	"	"	274	35	13	58	84	29	51	4	3
35	"	"	610	35	12	05	84	19	49	1	5
36	NC	Graham	354	35	26	58	83	56	37	3	1
37	"	"	945	35	24	59	83	58	25	10	5
38	"	"	1476	35	22	52	83	59	03	33	1
39	NC	Graham	1622	35	22	25	83	59	32	13	
40	"	Cherokee	640	35	12	02	84	13	57	1	4
41	TN	Monroe	287	35	32	09	84	11	37		6
42	"	"	280	35	29	26	84	12	21		5
43	"	"	259	35	29	23	84	11	08		2
44	"	"	274	35	28	56	84	12	54		17[a]
45	"	"	598	35	24	22	84	05	19		2
46	TN-NC	Monroe-Graham	1372	35	20	54	84	02	17	48[b]	14
47	TN	Monroe	1213	35	20	48	84	03	41	40[b]	14
48	"	"	1326	35	20	44	84	02	53	49[b]	10
49	"	"	427	35	20	17	84	14	03		1
50	"	"	518	35	19	57	84	08	34		1
51	TN-NC	Monroe-Cherokee	610	35	13	28	84	17	25		1
52	TN	Polk	244	35	13	23	84	31	06		1
53	"	"	348	35	10	33	84	19	30		1
54	NC	Swain	598	35	27	40	83	48	42		2
55	"	"	1280	35	17	51	83	41	38		31
56	"	Graham	622	35	17	40	83	53	30		28
57	"	Macon	707	35	12	36	83	30	34		24
58	"	Cherokee	512	35	09	45	84	11	10		3
59	"	Clay	1189	35	06	53	83	46	25		30

Locality No.	State	County	Elevation (m)	North latitude			West longitude			*P. aureolus*	*P. glutinosus*	*P. teyahalee*	*P. jordani*
				°	′	″	°	′	″				
60	TN	Knox	265	35	53	30	83	57	09		25		
61	"	Blount	348	35	39	56	83	47	04		6		
62	"	"	1037	35	32	09	83	52	48		10		
63	"	"	421	35	29	58	83	56	00		1		
64	"	Monroe	259	35	37	13	84	15	28		5		
65	"	"	262	35	36	43	84	12	07		5		
66	"	"	290	35	33	53	84	05	40		5		
67	"	"	293	35	28	08	84	13	50		7		
68	"	"	293	35	27	05	84	14	12		3		
69	"	"	311	35	23	33	84	21	22		5		
70	"	"	320	35	20	27	84	23	42		5		
71	"	"	485	35	17	53	84	27	21		4		
72	"	"	372	35	17	20	84	26	36		4		
73	"	McMinn	262	35	21	01	84	32	53		5		
74	"	"	628	35	20	04	84	24	29		9		
75	"	Polk	573	35	16	02	84	29	37		29		
76	"	"	317	35	15	22	84	27	40		23		
77	TN	Polk	274	35	12	11	84	30	50		7		
78	"	"	274	35	06	53	84	34	32		5		
79	"	"	268	35	05	26	84	43	44		5		
80	"	"	640	35	03	09	84	30	52		29		
81	NC	Swain	591	35	28	23	83	55	13		5		
82	"	Cherokee	512	35	08	32	84	11	01		12		
83	"	"	524	35	07	03	84	14	28		13		
84	"	"	494	35	03	40	84	09	49		5		
85	GA	Murray	351	34	56	39	84	42	43		7		

[a]hybrids with *P. glutinosus*

[b]hybrids with *P. jordani*

Ellis Branch of Spring Creek, near Springtown, in northern Polk County, Tennessee, with no genetic evidence of current hybridization between them (see below).

At 28 of the 31 *P. aureolus* localities, white-spotted *P. glutinosus* has been taken in sympatry. Highton (1972) pointed out that the name *Plethodon jordani teyahalee* Hairston (1950) is available for the latter form. The population at the type locality, Teyahalee Bald, Graham-Cherokee County line, North Carolina, is probably of hybrid origin, but is much more like white-spotted *P. glutinosus* than *P. jordani* (Highton 1970). As shown below, this form occurs sympatrically with both *P. aureolus* and *P. glutinosus* at locality 4, where apparent reproductive isolation exists between all three forms. On the basis of this evidence, I suggest that the white-spotted form should also be recognized as a distinct species, *P. teyahalee.* Its distribution and genetic relationships will be discussed in a later paper.

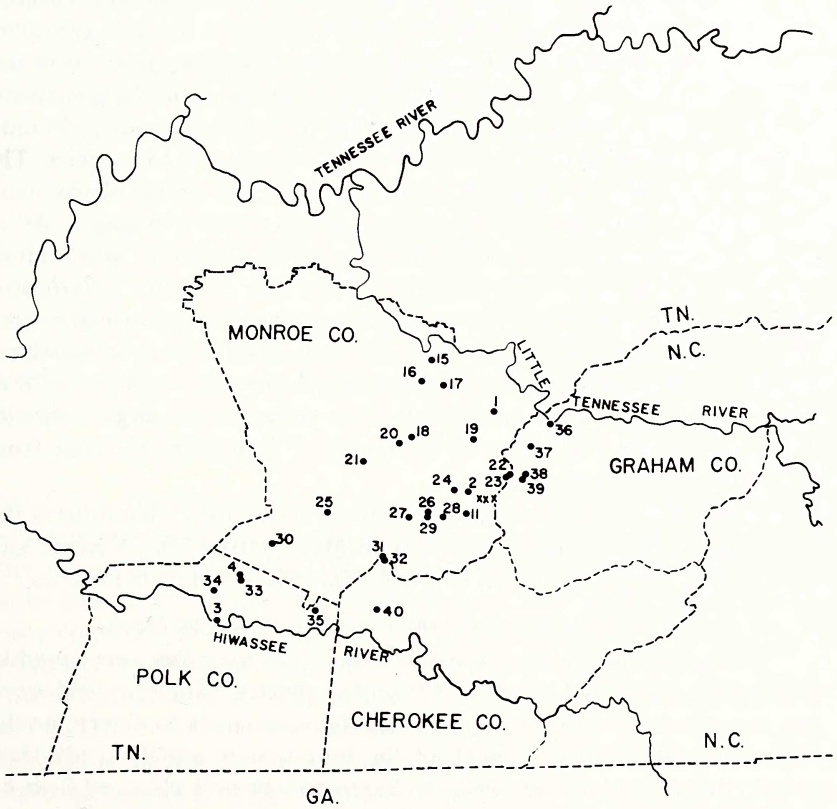

Fig. 1. Distribution of *P. aureolus* in southwestern North Carolina and southeastern Tennessee between the Hiwassee and Little Tennessee rivers. Crosses represent localities (46-48) where *P. aureolus* and *P. jordani* hybrids occur.

Plethodon jordani, another light-chinned species, occurs at higher elevations in the Unicoi Mountains (Highton 1962, 1970). An electrophoretic analysis of geographic genetic variation in *P. jordani* and *P. teyahalee* in the southern Appalachian Mountains by Peabody (1978) showed that the latter species is more closely related to some populations of *P. jordani* than it is to most other *P. glutinosus. Plethodon jordani* and *P. teyahalee* hybridize extensively in a number of contact zones (Highton 1970; Highton and Henry 1970; Peabody 1978), including the entire periphery of the range of *P. jordani* in the Unicoi Mountains, but in many areas the two species overlap extensively without evidence of hybridization. Although we have no evidence of current hybridization between *P. aureolus* and *P. teyahalee,* a transect through the only known contact zone between *P. jordani* and *P. aureolus* on Sassafras Ridge provided evidence of hybridization between these two species (unpublished data).

The small size of *P. aureolus* is one of its most distinctive features. As discussed in Duncan and Highton (1979), size is a difficult character to use in salamander taxonomy. In *Plethodon,* however, mean adult size and maximum length are sometimes quite consistent among genetically closely related populations. At the type locality of *P. aureolus,* 356 individuals were collected for a study of the life history of the species. The largest specimen is 67 mm from snout to anterior angle of the vent. Only three other individuals are over 61 mm in snout-vent length. All of the other species of southern Appalachian large *Plethodon* attain much larger sizes (Highton 1970). The mean adult size of both *P. glutinosus* and *P. teyahalee* is usually at least 70 mm and large adults are often over 80 mm (record size, a *P. teyahalee* from Davis Ridge in the Great Smoky Mountains, Sevier Co., Tennessee, 94 mm). In a sample of 78 *P. teyahalee* from the type locality of *P. aureolus* the 10 largest females range from 75-90 mm (mean 81.6) and the 10 largest males range from 74-90 mm (mean 78.5).

I suggest that an appropriate common name for *P. aureolus* is the Tellico salamander. Tellico Plains is located centrally in its range and much of this region is drained by the Tellico River and its tributaries.

Electrophoretic Genetic Analysis of Proteins.

Once collections from the type locality of *P. aureolus* were found to differ genetically from all other *Plethodon* species, extensive field work was done in the Unicoi Mountains and adjacent areas to determine the geographic distribution of each of the four species and to study their geographic and genetic interactions. Salamanders were obtained from 84 localities, and individuals from each of these were compared using the same 22 genetic loci and methods described in Highton and MacGregor (1983). When compared with a sample of *P. glutinosus* from near the type

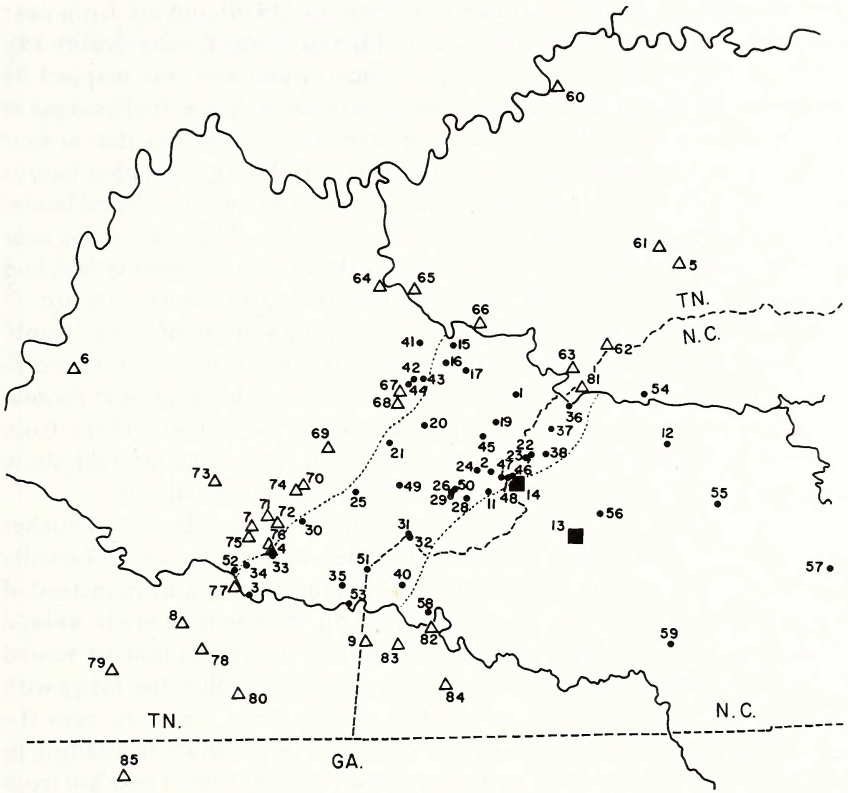

Fig. 2. Sites of samples of *P. glutinosus* (hollow triangles), *P. teyahalee* (solid circles) and *P. jordani* (solid squares) identified electrophoretically in southwestern North Carolina, southeastern Tennessee and northern Georgia. Dotted lines outline known range of *P. aureolus*.

locality in New Jersey (locality 10), 32 samples are genetically very similar and are referred to this species. All are from areas west, north or south of the range of *P. aureolus* (Fig. 2) and represent large, brassy-spotted animals similar in appearance to those from New Jersey, although some have chins that are much lighter than those in northern populations of *P. glutinosus*. *Plethodon teyahalee* was found at 44 localities, and at 28 of these *P. aureolus* was taken in sympatry. *Plethodon teyahalee* occurs at low and intermediate elevations east of the range of *P. glutinosus* (Fig. 2). Since *P. teyahalee* may be distinguished from the other two species by its color pattern in life, its distribution west of the French Broad River is probably accurately indicated by the map in Highton (1970, Fig. 5).

Two samples from populations of Unicoi Mountain *P. jordani* are included in order to compare this species with the three other forms. These

are the same salamanders studied by Peabody (1978) and are from near Junction, on Snowbird Creek (locality 13) and Johns Knob (locality 14). The distribution of *P. jordani* in the Unicoi Mountains was mapped by Highton (1970) and its range supposedly includes all of the higher areas of this mountain range. It was therefore surprising to discover that at four localities in the northernmost part of the Unicoi Mountains, populations resembling *P. jordani* in coloration (no dorsal spotting and reduced lateral spotting), are assigned genetically to *P. aureolus*. These are from near Cherry Log Gap (localities 22 and 23), Naked Ground (locality 38), and Stratton Bald (locality 39). Thus the northernmost known sites for *P. jordani* in the Unicoi Mountains are now in the vicinity of Johns Knob. Highton (1970) called attention to the apparent hybridization between *P. jordani* and *P. teyahalee* all around the periphery of the range of *P. jordani* in the Unicoi Mountains. Two transects reported by Peabody (1978) (from Johns Knob west along the North River, and from Junction east along Snowbird Creek) have confirmed this hybridization genetically.

At some of the 84 localities few animals were collected. Voucher specimens were preserved from all of the sites, and tissue samples (usually viscera and tail muscle) from some were used for electrophoresis instead of the material used in my previous work on *Plethodon* (whole animal homogenates). The three general protein loci usually cannot be scored from homogenates of viscera and tail muscle. Rather than use many with small sample sizes and/or incomplete genetic data, I present here the results of a complete genetic analysis of only 17 populations in addition to the sample of *P. glutinosus* from New Jersey (locality 10). These are from scattered sites throughout the local ranges of the four species: 4 *P. aureolus* (localities 1-4), 6 *P. glutinosus* (localities 4-9), 5 *P. teyahalee* (localities 1, 2, 4, 11, 12) and 2 *P. jordani* (localities 13 and 14). Three species are sympatric at locality 4, and *P. aureolus* and *P. teyahalee* are sympatric at localities 1 and 2. Material from all other localities shows no unusual genetic variation beyond that observed in the 18 samples for which complete genetic analysis is presented.

Table 2 provides the frequency data of genic variation of 18 populations from 14 localities. Of the 22 presumed genetic loci evaluated, 3 (Mdh-1, Pep, and Pt-3) show no variation. Three loci are monomorphic except for a single population: α-Gpd has a rare slower allelomorph in *P. glutinosus* (.02) at locality 8, Gdh has a slower allelomorph (.29) in *P. teyahalee* at locality 1, and Mdh-2 has a rare faster allelomorph (.02) in *P. aureolus* at locality 2. Table 3 gives Nei standard genetic distances (*D*) and normalized identity of genes (*I*) (Nei 1972) for all comparisons and the mean heterozygosity (*H*) estimated from allelomorph frequencies. The *I* values are clustered by the UPGMA method (Sneath and Sokal 1973) in a phenogram in Figure 3.

Table 2. Genic variation within and among samples of *Plethodon*.

	P. aureolus				*P. glutinosus*							*P. teyahalee*					*P. jordani*	
Locality:	1	2	3	4	5	6	7	8	9	10	4	1	2	11	12	4	13	14
Locus n:	36	30	33	5	30	5	28	32	30	30	6	39	32	27	35	13	30	36
A1b	c	b(.10)	b	b	b	b	b	b(.95)	b	b	b	a	a	a(.69)	a(.97)	a(.92)	b	a(.03)
	(n=92)	c(.90)	(n=37)		(n=53)	(n=9)	(n=33)	d(.05)		(n=78)		(n=48)	(n=30)	c(.31)	c(.03)	b(.08)	(n=37)	b(.94)
								(n=31)						(n=26)				c(.04)
																		(n=39)
Est	k(.04)	m(.03)	c(.02)	c(.10)	l(.48)	l(.70)	h(.20)	h(.03)	c(.02)	l(.39)	c(.17)	c	a(.03)	c(.80)	c(.99)	c(.96)	b(.15)	c(.19)
	l(.07)	0(.62)	m(.11)	h(.10)	p(.52)	p(.30)	l(.55)	l(.89)	f(.03)	p(.61)	h(.33)		c(.88)	f(.02)	j(.01)	l(.04)	c(.07)	d(.04)
	p(.89)	p(.10)	j(.03)	k(.10)			o(.05)	p(.05)	g(.02)	(n=27)	i(.08)		o(.09)	k(.02)			e(.02)	f(.11)
		r(.25)	k(.02)	m(.60)			p(.20)	s(.02)	i(.07)		l(.33)			p(.15)			f(.33)	k(.01)
			l(.35)	p(.10)				t(.02)	l(.60)		p(.08)			r(.02)			h(.03)	p(.24)
			n(.20)						p(.05)								l(.22)	q(.24)
			p(.29)						s(.18)								p(.17)	r(.15)
									t(.03)								t(.02)	s(.01)
Fum	a(.34)	a(.03)	b	b	b(.67)	b	b	b	b	a(.02)	b	b	b	a(.02)	b	b	b	b
	b(.66)	b(.97)			c(.33)					b(.98)				b(.98)				
	(n=35)				(n=27)													
Got-1	b(.03)	c	c	c	c	c	c	c(.94)	c(.97)	a(.10)	b(.17)	b	b(.98)	b(.96)	b(.97)	b(.69)	b(.47)	b(.33)
	c(.97)							d(.06)	d(.03)	c(.90)	c(.83)		c(.02)	c(.04)	c(.03)	c(.23)	c(.24)	c(.13)
	(n=33)															d(.08)	e(.29)	c(.54)
Got-2	b	b	a(.02)	a(.70)	b	b	a(.20)	b	b	b	a(.58)	b	b	b	b	b	b	b
	(n=33)	d	b(.98)	b(.30)			b(.77)				b(.25)							
							c(.04)				c(.17)							
Idh-1	a(.48)	a	a(.74)	a	a	a	a(.96)	a(.47)	a(.40)	a	a	a(.90)	a(.83)	a(.81)	a(.97)	a(.96)	a(.38)	a(.67)
	b(.52)		b(.15)				b(.04)	b(.53)	b(.60)			b(.10)	b(.17)	b(.19)	b(.03)	b(.04)	b(.62)	b(.33)
	(n=33)		c(.11)				(n=26)											
Idh-2	a(.03)	b(.38)	b(.05)	b(.20)	b(.88)	b	b(.96)	b(.42)	b(.10)	b(.72)	b	b	b(.97)	b	b(.96)	b	b.(95)	b(.85)
	b(.29)	c(.62)	c(.95)	c(.80)	d(.12)		c(.04)	c(.58)	c(.90)	d(.28)			c(.03)		c(.04)		d(.05)	d(.15)
	c(.68)														(n=34)		(n=29)	
	(n=35)																	
Ldh	b	b	a(.02)	b	a(.53)	b	a(.30)	a(.13)	b	b	a(.17)	b	b	b	b	b	b	b
(heart)			b(.98)		b(.47)		b(.70)	b(.88)			b(.83)							
Ldh	c(.86)	c(.90)	c(.98)	c	d	d	d	d	d	d	d	d(.36)	a(.02)	e	e	e	b(.02)	b(.22)
(muscle)	e(.14)	e(.10)	e(.02)									e(.64)	e(.98)				e(.98)	e(.78)
		(n=29)										(n=38)						

Table 2. Genic variation within and among samples of *Plethodon* (cont'd).

	P. aureolus				*P. glutinosus*							*P. teyahalee*					*P. jordani*	
Locality:	1	2	3	4	5	6	7	8	9	10	4	1	2	11	12	4	13	14
Locus n:	36	30	33	5	30	5	28	32	30	30	6	39	32	27	35	13	30	36
Lap	a	a (n=29)	a	a	a	a(.70) b(.30)	a(.98) b(.02)	a	a	a (n=28)	a	a (n=32)	a(.97) b(.02) c(.02)	a(.98) c(.02)	a	a	a(.78) c(.22)	a(.86) c(.14)
Pgi	b(.01) d(.99)	d (n=29)	d	a(.10) d(.90)	d	d	d	c(.02) d(.98)	a(.02) d(.98)	d	a(.08) d(.92)	a(.64) d(.36)	a(.58) d(.42)	a(.81) d(.19)	a(.43) d(.57)	a(.62) d(.38)	a(.03) d(.97) (n=29)	a(.18) d(.82)
Pgm	a	a(.83) b(.17)	a	a	a(.80) b(.20)	a	a	a	a	a	a	a (n=38)	a	a	a	a	a(.93) b(.07)	a(.88) b(.13)
Pt-1	b (n=33)	a(.60) b(.40) (n=29)	b	b	a	a	a	a	a	a	a(.92) b(.08)	a	a	a	a	a	a	a(.91) b(.09) (n=34)
Pt-2	d (n=33)	b(.12) d(.88) (n=29)	b(.02) d(.98) (n=30)	b(.10) d(.90)	a (n=26)	a	a	a	a	a	a(.92) b(.08)	b	b	b	b	a(.08) b(.92)	b(.71) c(.29) (n=29)	b(.98) c(.02) (n=27)
6-Pgd	a(.02) b(.76) d(.23) (n=33)	b(.95) d(.05) (n=28)	b(.17) d(.83)	b(.50) d(.50)	d	b	b(.73) d(.27)	b(.10) d(.90) (n=30)	d	d (n=27)	b(.58) d(.42)	b (n=32)	b(.89) d(.11)	a(.93) b(.07)	b	b	b(.38) c(.16) d(.47) (n=29)	b(.42) c(.10) d(.49)
	a(.01) b(.03) c(.05) d(.05) e(.10) f(.09) g(.10) h(.19) i(.09) j(.20) k(.04) m(.03) n(.01) p(.01) g(.01) (n=76)	e(.35) g(.15) j(.40) k(.08) q(.02) (n=26)	b(.03) e(.97) (n=37)	e	c(.25) d(.03) e(.23) f(.13) g(.03) i(.10) l(.05) m(.15) q(.05) (n=20)	c(.22) e(.50) i(.28) (n=9)	c(.26) f(.12) g(.12) h(.14) i(.11) j(.02) k(.24) (n=33)	c(.19) d(.02) f(.31) g(.02) i(.21) l(.21) o(.04) (n=24)	c(.07) f(.23) g(.02) i(.07) j(.05) l(.45) p(.12)	d (n=78)	a(.17) c(.08) d(.08) f(.08) g(.08) h(.08) k(.42)	a(.95) b(.05) (n=67)	a(.67) b(.12) c(.08) d(.02) i(.12) (n=30)	a(.80) b(.17) h(.04)	a(.99) g(.01)	a(.88) k(.08) m(.04)	a(.06) b(.12) c(.33) h(.36) i(.12) (n=33)	a(.32) b(.14) c(.01) f(.04) h(.01) i(.33) j(.12) k(.03) (n=38)

Table 3. Normalized identity of genes *(I)* above diagonal and genetic distance *(D)* below diagonal for all pairs of samples. Genic heterozygosity *(H)*, estimated from allele frequencies, is given at bottom of table.

	P. aureolus				*P. glutinosus*							*P. teyahalee*					*P. jordani*	
Locality	1	2	3	4	5	6	7	8	9	10	4	1	2	11	12	4	13	14
1		.92	.88	.85	.69	.72	.72	.73	.73	.71	.70	.63	.65	.67	.65	.66	.72	.72
2	.08		.87	.86	.74	.80	.79	.76	.75	.75	.77	.71	.73	.73	.73	.74	.76	.78
3	.13	.14		.95	.76	.76	.75	.80	.81	.77	.74	.59	.61	.60	.61	.62	.73	.74
4	.17	.15	.05		.72	.75	.75	.74	.75	.73	.78	.61	.62	.61	.62	.64	.71	.73
5	.37	.30	.27	.33		.92	.95	.94	.91	.95	.92	.66	.67	.66	.66	.69	.81	.80
6	.32	.22	.28	.29	.09		.97	.91	.87	.91	.94	.73	.73	.72	.73	.75	.82	.82
7	.32	.23	.29	.28	.05	.03		.94	.90	.93	.98	.73	.73	.72	.73	.75	.83	.83
8	,32	.28	.23	.30	.06	.09	.06		.99	.93	.91	.67	.68	.66	.66	.69	.83	.81
9	.32	.29	.21	.29	.10	.14	.11	.01		.91	.88	.64	.65	.64	.64	.66	.81	.79
10	.34	.29	.27	.32	.05	.10	.08	.07	.09		.92	.68	.68	.67	.68	.70	.81	.82
4	.36	.26	.30	.25	.08	.07	.02	.10	.13	.09		.74	.74	.73	.74	.76	.82	.83
1	.46	.34	.53	.50	.41	.31	.32	.40	.44	.39	.29		.99	.98	.99	.98	.81	.86
2	.42	.32	.49	.47	.40	.31	.32	.39	.42	.38	.30	.01		.99	.99	.99	.86	.89
11	.40	.31	.51	.49	.42	.32	.33	.41	.45	.39	.31	.02	.01		.98	.99	.85	.88
12	.43	.32	.50	.47	.41	.32	.32	.41	.45	.39	.30	.01	.01	.02		.99	.84	.88
4	.41	.30	.48	.45	.38	.28	.29	.38	.42	.36	.27	.02	.01	.01	.01		.84	.88
13	.33	.28	.31	.35	.22	.19	.18	.19	.21	.21	.19	.20	.16	.17	.18	.17		.97
14	.32	.25	.30	.32	.22	.20	.19	.21	.24	.20	.19	.16	.12	.13	.13	.13	.03	
H	.14	.15	.08	.10	.13	.07	.13	.12	.09	.05	.16	.07	.09	.09	.04	.07	.20	.22

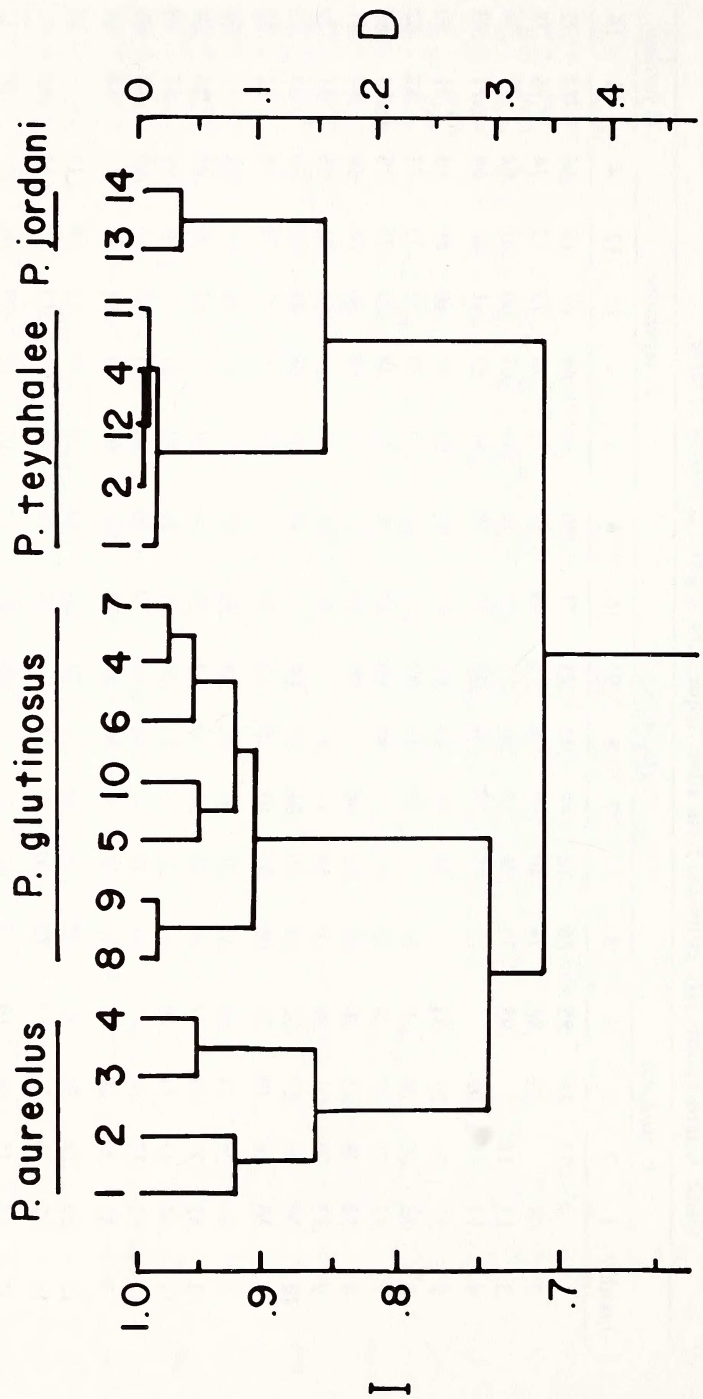

Fig. 3. UPGMA phenogram of *I* and *D* values of 18 populations of 4 species of *Plethodon* from 14 localities. Cophenetic correlation coefficient is .89.

The 18 samples cluster into 4 groups, each representing a taxonomic species. There is very little geographic genetic variation within *P. teyahalee,* but there is a considerable amount in both *P. glutinosus* and *P. aureolus*. Indeed, some samples within both of the latter species are as different genetically as are some comparisons of *P. teyahalee* and *P. jordani*. The southwestern populations of *P. jordani* from the Unicoi and Nantahala Mountains are genetically very similar to *P. teyahalee* (Peabody 1978); in fact, these two species are more closely related than any other two species of *Plethodon* yet examined (see Highton and Larson 1979). It is therefore not surprising that they hybridize so extensively (Highton 1970).

Plethodon jordani is the most variable species (mean H=.21), *P. aureolus* (mean H=.12) and *P. glutinosus* (mean H=.11) are intermediate, while *P. teyahalee* (mean H=.07) is the least variable. Compared to more northern populations of *P. glutinosus* and *P. jordani* (Highton and MacGregor 1983), these southern samples are much more variable. The *P. glutinosus*, however, have slightly lower average H values than Arkansas and Oklahoma *P. glutinosus* (Duncan and Highton 1979).

In light of my unpublished evidence that *P. aureolus* and *P. jordani* hybridize at localities 46-48 on Sassafras Ridge (the only known area of contact between the two species), the proper taxonomic relationship between the two forms is difficult to decide. The average D of the 8 comparisons between the two forms (.31) is not very different from that of the 28 comparisons between *P. aureolus* and *P. glutinosus* (.29) or the 20 comparisons between *P. aureolus* and *P. teyahalee* (.43), two species with which *P. aureolus* is sympatric and is not known to hybridize. In the southwestern isolates of *P. jordani* (in the Great Smoky Mountains, Cowee Bald, the Nantahala Mountains, Cheoah Bald, and in the Unicoi Mountains), *P. jordani* is always a high altitude species, whereas *P. aureolus* is mostly a lower altitude form. The color pattern is very different (except in the northern Unicoi Mountains where hybridization between the two has occurred). None of the above mentioned populations of nearby *P. jordani* has as abundant lateral and dorsal yellow, white or brassy spotting. *Plethodon aureolus* is not significantly more similar genetically to adjacent samples of Unicoi Mountain *P. jordani* than it is to other populations of *P. jordani* throughout its range (Peabody 1978). The only similarity between *P. jordani* and *P. aureolus* is that they are both smaller than Appalachian populations of *P. glutinosus* and *P. teyahalee*. I therefore regard the interbreeding between the two on Sassafras Ridge as a case of hybridization between species rather than intergradation between conspecific populations. Considering the very extensive hybridization between *P. jordani* and *P. teyahalee* throughout their contact zone in the Unicoi Mountains, it is curious that *P. teyahalee* does not appear to hybridize with the hybrid populations of *P. aureolus* and *P. jordani* on Sassafras Ridge.

The isolating mechanisms that keep *P. aureolus* from interbreeding with *P. teyahalee* apparently are present in the *aureolus-jordani* hybrids in sufficient degree to prevent the usual interbreeding of *P. jordani* and *P. teyahalee* at all 3 Sassafras Ridge sites (localities 46-48).

Since without genetic data it is extremely difficult to correctly identify many individuals of this complex of southern Appalachian large *Plethodon*, particularly some *P. aureolus* and *P. glutinosus*, there is a problem in assigning individuals to species before examining the genetic data. After a long search for a site where the two widely sympatric species, *P. teyahalee* and *P. aureolus*, contact the parapatric species, *P. glutinosus*, a localilty was discovered along Ellis Branch, near Springtown, Polk County, Tennessee (locality 4), where the three forms are sympatric. Nei genetic identities between all 24 individuals from this localilty are clustered in a UPGMA phenogram (Fig. 4). The results clearly show that the 24 animals are separable into three groups consisting of 13 *P. teyahalee*, 5 *P. aureolus* and 6 *P. glutinosus*. Four additional very small animals from locality 4 were also examined at some of the diagnostic loci and were identified as *P. teyahalee*, but are not included in the genetic analysis because of incomplete genetic data for several loci. Each of these samples clusters with others of its own species (Fig. 3), and only the *P. glutinosus* sample has a higher than average *H* value (Table 3). The allelic data in Table 4 indicate that there is only one locus (Ldh-muscle) in which there are fixed differences between all three species. At the other differential loci, sometimes two of the species have identical electromorphs and sometimes there are rare electromorphs of the same kind found in one or both of the other species. This latter pattern of variation is also present in sympatric populations of *P. glutinosus* and *P. kentucki* (Highton and MacGregor 1983) and could result from inheritance of the same electromorphs from their common ancestor, or occasional hybridization between the species after complete differentiation had occurred. The relationships in Figures 3 and 4 and the data in Tables 2, 3 and 4 are considered strong evidence for the recognition of all three forms as distinct species. The Ldh-muscle data clearly show that there is not a single F_1 hybrid between any of the three species at the Ellis Branch locality, as does the pattern of variation at the other differential loci.

I have no explanation as to why in three cases an electromorph from another species appears as a rare homozygote instead of in the expected heterozygous condition [*P. teyahalee* #9, Alb; *P. aureolus* #21, Idh-2; and *P. glutinosus* #16, Ldh (heart)] (see Table 4).

The Pep electromorphs of *P. aureolus* are faster than those of the other two species at locality 4 and are indicated as different in Table 4. This difference could not be consistently detected on comparison gels of samples of the three species at other localities and is therefore not regarded as a

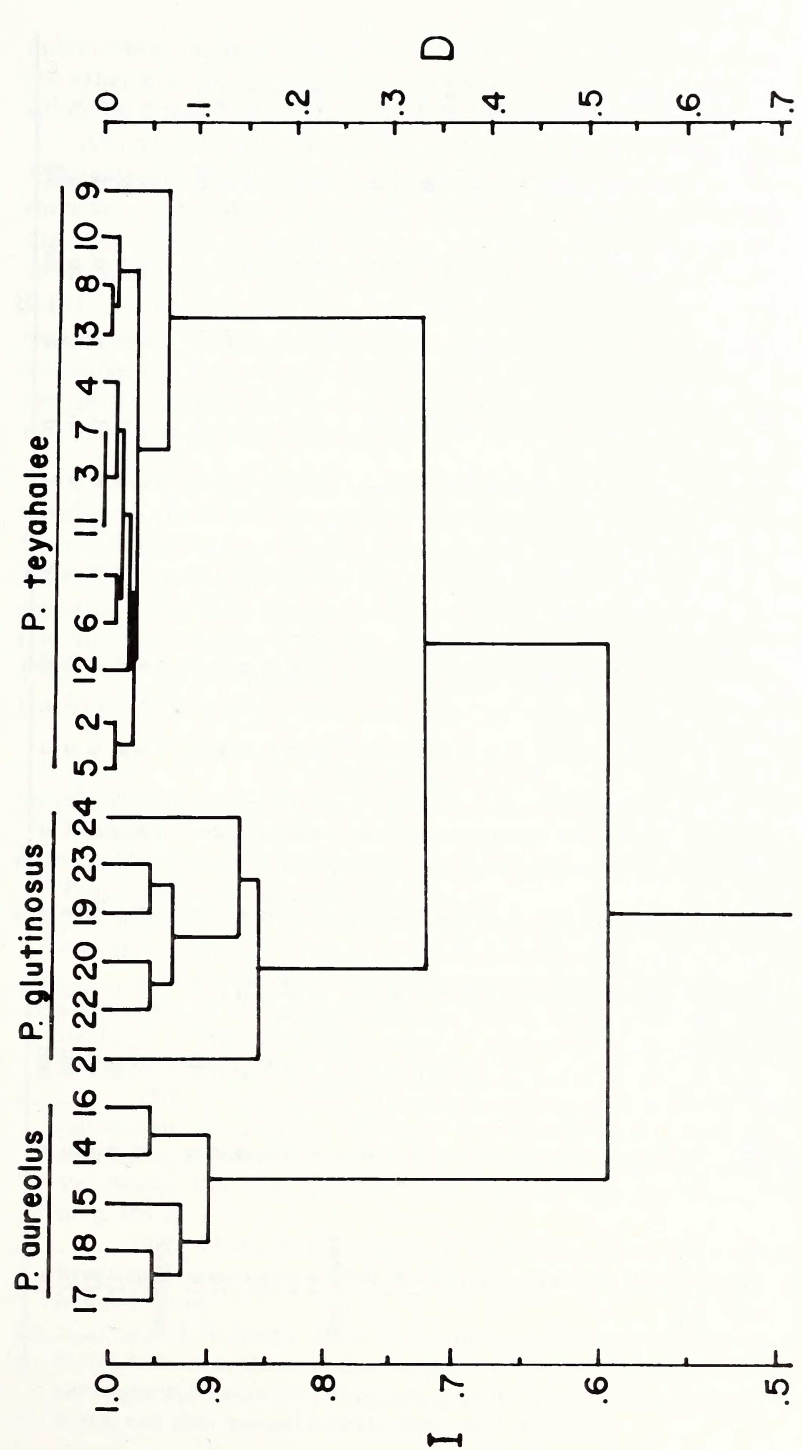

Fig. 4. UPGMA phenogram of *I* and D values of 24 individuals from locality 4.

Table 4. Genotypes of 24 individuals from locality 4 for the 14 variable loci.

Individual No.	Locus													
	Alb	Est	Got-1	Got-2	Idh-1	Idh-2	Ldh (heart)	Ldh (muscle)	Pep	Pgi	Pt-1	Pt-2	6-Pgd	Trf
teyahalee														
1	a	c	bc	b	a	b	b	e	b	ad	a	ab	b	a
2	a	c	b	b	a	b	b	e	b	ad	a	b	b	ak
3	a	c	bc	b	a	b	b	e	b	ad	a	b	b	a
4	a	cl	bc	b	a	b	b	e	b	ad	a	b	b	a
5	a	c	bd	b	a	b	b	e	b	ad	a	b	b	ak
6	a	c	b	b	a	b	b	e	b	ad	a	ab	b	a
7	a	c	bc	b	a	b	b	e	b	ad	a	b	b	a
8	a	c	bc	b	a	b	b	e	b	a	a	b	b	a
9	b	c	bd	b	a	b	b	e	b	ad	a	b	b	a
10	a	c	b	b	a	b	b	e	b	a	a	b	b	am
11	a	c	bc	b	a	b	b	e	b	ad	a	b	b	a
12	a	c	b	b	ab	b	b	e	b	ad	a	b	b	a
13	a	c	b	b	a	b	b	e	b	a	a	b	b	a
glutinosus														
14	b	l	c	ac	a	b	b	d	b	d	a	a	b	ak
15	b	hl	bc	ac	a	b	b	d	b	d	a	a	bd	fk
16	b	hp	c	ab	a	b	a	d	b	d	a	ab	d	ak
17	b	h	c	a	a	b	b	d	b	d	a	a	bd	hk
18	b	cl	c	a	a	b	b	d	b	d	ab	a	b	gk
19	b	ci	bc	b	a	b	b	d	b	ad	a	a	bd	cd
aureolus														
20	b	m	c	b	a	c	b	c	a	d	b	d	d	e
21	b	m	c	a	a	b	b	c	a	d	b	d	b	e
22	b	kp	c	ab	a	c	b	c	a	d	b	d	d	e
23	b	cm	c	a	a	c	b	c	a	ad	b	d	b	e
24	b	hm	c	a	a	c	b	c	a	d	b	bd	bd	e

polymorphic locus in Table 2. Thus the *D* values between *P. aureolus* and the other two species indicated in Figure 4 are slightly higher than they would be if this Pep difference had not been detected (see Fig. 3.)

Although there is little or no evidence for hybridization between *P. teyahalee* and *P. glutinosus* in the samples from locality 4, at locality 44 there are 4 individuals in a sample of 17 that have dorsal spots of intermediate coloration. In addition, there is a low frequency of *P. glutinosus* electromorphs at all of the loci that differentiate the two species (Alb, Est, Ldh (muscle), Pt-2, and Trf). This is interpreted as evidence for hybridization between the two species at this locality. It is not surprising that *P. teyahalee* and *P. glutinosus* hybridize in some areas and not in others, since this same pattern occurs between *P. teyahalee* and *P. jordani* in the southern Appalachian Mountains.

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